Sporicidal Activity of Heated Dolomite Powder against *Bacillus subtilis* Spore

Shogo Yasue1), Jun Sawai2), Mikio Kikuchi2)

1) Graduate School in Kanagawa Institute of Technology, Department of Applied Chemistry, 2) Faculty of Applied Bioscience, Kanagawa Institute of Technology, 1030 shimo-ogino, Atsugi, kanagawa 243-0292, Japan  *sawai@bio.kanagawa-it.ac.jp*

**Abstract**

The heated dolomite powder slurry was investigated for the sporicidal activity against *Bacillus subtilis* spore. *B. subtilis* spore used in this study did not decreased at pH 1 or 13 for 2 h, indicating that the spores had a sufficient resistance. However, the dolomite powder heated at 1000℃ for 1 h could kill *B. subtilis* spore, even the pH of the slurry was 12.7. The dolomite powder heated at 700-750℃ did not exhibit the sporicidal activity. The sporicidal activity appeared when the dolomite powder heated at 800℃ or higher, and the raise in the heating temperature increased with the sporicidal activity. This temperature corresponded to that of generation of CaO. On the other hand, MgO did not contribute to the sporicidal activity of the heated dolomite powder. To elucidate the sporicidal mechanism of the heated dolomite powder against *Bacillus subtilis* spore, the generation of active oxygen from the slurry was examined by a chemiluminescence analysis. The luminescence intensity has increased when the slurry concentration rose. The results suggested that active oxygen species generated from the heated dolomite powder were associated with the sporicidal activity.

**Key words**: dolomite, calcium oxide (CaO), *Bacillus subtilis* spore, antibacterial activity, sporicidal activity, active oxygen

**1. INTRODUCION**

The dolomite is a natural ore that consists of calcium carbonate (CaCO₃) and magnesium carbonate (MgCO₃). Dolomite is primarily used for refinement of steel, soda of glass, aggregate of road and concrete, and is also used as a soil conditioner and a supplement. The main component of dolomite, CaCO₃ and MgCO₃, become calcium oxide (CaO) and magnesium oxide (MgO), respectively by heat treatment, and as-prepared dolomite comes to be antibacterial1). Recently, problems concerning public health and food hygiene, such as large-scale food poisoning (SARS, bird influenzas, and new influenzas), happened frequently2). Then, the heated dolomite shows the antivirus activity3) as well as the antibacterial activity and is expected for controlling of microbes and these infections.

Moreover, killing of the spore is one of the still serious problems in food hygiene. The spore is a cell of dormancy forms that a part of bacteria is received stimulation by an external change such as hunger and dryness, and in formed in the cell. The typical
bacteria that forms the spore are *Bacillus* sp. of the aerobes bacteria and *Clostridium* sp. of obligate anaerobes bacteria. The spore is a dormant structure in the severe environment for a long term. The spore has the high resistance more remarkably than usual vegetative cells against various physicochemical stresses such as heat, dryness, radiation, chemicals, and acids.

The heated dolomite powder exhibited the antibacterial activity against the vegetative cell of bacteria. However, the action of the heated dolomite on the bacterial spore has not been elucidated yet. In this study, we investigated the sporicidal activity of the heated dolomite powder against *B. subtilis* spores.

2. MATERIALS AND METHODS

2.1. Treatment of the heated dolomite powder slurry

*Bacillus subtilis* spore (Eiken Chemical Co., Ltd.) was used as the test organism. The dolomite powder was heated to temperature from 700 to 1000°C for 1 h, and a powder slurry was prepared by suspending the dolomite powder in sterilized physiological saline. The spore suspension was pipetted into the slurry (37°C). Periodically, a sample was withdrawn and incubated with nutrient agar (Eiken Chemicals). Colonies were counted after incubation at 37°C for 24 h.

2.2. Chemiluminescence analysis

The active oxygen generated from the dolomite powder was detected as the chemiluminescence response of the reaction between luminol and active oxygen. For measurements, Micro Plate Reader Mithras LB09470 (Berthold Technologies) was used. The dolomite powder slurry of 100 μL was pipetted into the well of 96 wells microplate, and the microplate was set in the apparatus. The chemiluminescence reaction was initiated by the addition of 50 μL of 7 mM luminol (Nacalai Tesque, Inc.) to the slurry from the dispenser, and the chemiluminescence response was recorded. When the effects of antioxidative enzymes were examined, 0.1 mg/mL superoxide-dismutase (SOD) or 0.1 mg/mL catalase (Wako Pure Chemical Industries, Ltd.) solution of 25 μL was added to the wells from the dispenser before the addition of luminol.

3. RESULT & DISCUSSION

3.1. Tolerance of the *B. subtilis* spore to acid or alkali treatment

Fig. 1 shows the effect of treatment of acid (pH 1) or alkali (pH 13) for 120 min against *B. subtilis* spore used in this study. The experimental temperature is 37°C. The ordinate shows the survival ratio. *B. subtilis* spore has not almost decreased for 120 min in sulfuric acid solution of pH 1 or sodium hydroxide solution of pH 13. Therefore, the spore used in this study has the sufficient resistance to strong acid or strong alkali.

3.2. Sporicidal activity of the heated dolomite powder

Fig. 2 shows the sporicidal activity of dolomite powder heated at 1000°C for 1 h against *B. subtilis* spore. The sporicidal activity of the heated dolomite powder slurry at the slurry concentration of 3 mg/mL (pH 12.7) is higher than that of NaOH of pH 13. The effect has increased when the slurry concentration rose.
The heated dolomite powder could kill the *B. subtilis* spore at the room temperature.

![Graph showing thermogravimetric curve](image)

The thermogravimetric (TG) curve of the heated dolomite powder is shown in Fig. 4. A weight decrease started from about 700°C, changed the slope once at about 800°C, and ended at 890°C. The result of the TG curve was corresponding to the result of Fig. 3 well. In addition, the powder slurry of only CaO also had the sporicidal activity. These results suggested that sporicidal activity generated from the heated dolomite powder was due to generation of CaO.

![Graph showing temperature vs. weight change](image)

3.3. Detection of active oxygen by the chemiluminescence analysis

In the previous study, it was confirmed that the active oxygen species (especially, superoxide O$_2^-$) was generated from the CaO and MgO powder slurries. The sporicidal activity of the heated dolomite was much stronger than that of a simple alkali treatment at the same pH level (Figs 1 and 2). In the action mechanism of the dolomite, it is clear that they have...
the factor besides the pH in addition to the alkaline effect.

Fig. 5 shows the results of measurements of the active oxygen generated from the heated dolomite powder slurry by the chemiluminescence analysis. The ordinate shows the luminescence intensity (counts/s). Increases in powder concentrations enhanced the chemiluminescence response. To prove that the luminescence responses were due to the active oxygen species generated from the heated dolomite powder, the effects of antioxidative enzymes, such as super oxidedismutase (SOD) and catalase, on the chemiluminescence response were examined. The addition of SOD or catalase remarkably inhibited the chemiluminescence responses of the heated dolomite powder slurry. The results suggested that the active oxygen species generated from the heated dolomite powder was associated with the sporicidal activity.

4. CONCLUSION

In this study, we obtained the following results and conclusions.

1) The heated dolomite powder could kill the B. subtilis spore at the room temperature. The spore used in this study has the sufficient resistance to strong acid or strong alkali. But, the sporicidal activity of the heated dolomite powder slurry at pH 12.7 is much higher than that of NaOH of pH 13.

2) The sporicidal activity generated from the heated dolomite powder was due to generation of CaO. Increases in the heating temperature caused the sporicidal activity at the heating temperature of 800°C or higher. This temperature corresponded to that of generation of CaO.

3) The generation of active oxygen from the heated dolomite powder slurry was observed by the chemiluminescence analysis. The results suggested that the active oxygen generated from the heated dolomite powder slurry was one of the factors of the sporicidal activity.

5. REFERENCE


(Received January 24, 2011; Accepted October 6, 2011)